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Tumor necrosis factor receptors (TNFRs) on T lymphocytes and soluble TNFRs in different clinical courses of sarcoidosis

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Summary
Introduction: The release of tumor necrosis factor (TNF-α) is increased in sarcoidosis patients. TNF-α exerts its effect by binding to specific cell surface receptors. There are only fragmentary data concerning the expression of tumor necrosis factor receptors (TNFRs) on bronchoalveolar lavage fluid (BALF) and peripheral blood (PB) lymphocytes. The aim of the study was to evaluate TNFRI (CD120a) and TNFRII (CD120b) expression on T cells and the level of soluble TNFRs in specimens of patients with different clinical manifestation and clinical outcome of sarcoidosis.
Material and methods: We examined 49 patients with newly diagnosed pulmonary sarcoidosis. TNFRI and TNFRII density on CD4+ and CD8+ BALF and PB cells surface was estimated using monoclonal antibodies and a flow cytometry technique. The level of TNFRs in PB serum and BALF cell culture supernatant (CCS) was measured using ELISA. Immunological analyses were also performed on PB samples collected from 10 healthy volunteers.
Results: The level of soluble TNFRI (sTNFRI) in PB serum was similar in sarcoidosis patients and healthy subjects, whereas the concentration of sTNFRII in serum was significantly higher in the sarcoidosis group ($P<0.001$). Patients without acute symptoms of sarcoidosis, patients with radiological stage II/III as well as patients with further disease progression showed a tendency to higher levels of sTNFRs in PB serum and lower levels of sTNFRs in BALF CCS compared to Löfgren syndrome and radiological stage I subjects, and patients with spontaneous resolution of sarcoidosis.
More than 80% of BALF and PB lymphocytes of sarcoidosis patients expressed both CD120a and CD120b antigens. The percentage of double-positive CD4+CD120a+ and CD4+CD120b+

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cells in PB was significantly higher ($P<0.005$) in sarcoidosis patients than in healthy subjects. The highest percentage of CD4+CD120a+ and CD4+CD120b+ lymphocytes in BALF was determined in patients with acute disease, and in PB of patients with further spontaneous improvement.
Conclusion: The evaluation of sTNFRs and TNFRs expression on T-helper cells may be useful in the estimation of sarcoidosis activity.

Introduction

Sarcoidosis is a systemic granulomatous disease of unknown origin that predominantly affects the lung. The disease is characterized by lymphocytic alveolitis and the formation of a noncaseating, epithelioid granuloma with the predominance of CD4+ Th cells and macrophages. The cells release chemokines and cytokines, which results in cellular proliferation and granuloma formation.[1]

Tumor necrosis factor (TNF-α) plays a key role in the pathogenesis of various infections and inflammatory diseases. The major source of TNF-α are activated monocytes (macrophages), though the cytokine is also released by T cells. Biological effects of TNF-α include local activation of vascular endothelium, an increase in vascular permeability, an increase in the expression of adhesion molecules on blood vessel endothelium, and an increase in class II MHC molecules expression.[2,3]

On the basis of its macrophage-stimulating capacity and inflammatory cell accumulation, TNF-α can be classified as a Th1-type, pro-inflammatory cytokine. The release of TNF-α is increased in the lung of patients with pulmonary sarcoidosis.[4,5] TNF-α initiates the development of giant and epithelial cells. It is responsible for granuloma formation in the lung. Chronic overexpression of TNF-α and INF-γ may lead to the progression of inflammation in chronic sarcoidosis patients.[6] The estimation of TNF-α released by alveolar macrophages may help to find a group of patients with an increased risk of disease prolongation and the development of unfavorable sarcoidosis course.[7]

TNF-α exerts its effect by binding to specific cell surface receptors. Two types of TNF receptors, 55-kD receptor (TNFRI, CD120a) and 75-kD receptor (TNFRII, CD120b), have been identified. Both types show high binding affinity of TNF-α. TNFRI is the main signaling receptor and is ubiquitous in human tissues, whereas TNFRII expression appears to be restricted to myeloid cells. These receptors are enzymatically cleaved from cell surface and form soluble TNF receptors: sTNFRI and sTNFRII. sTNFRs may inhibit TNF-α bioactivity, acting as TNF-α antagonists, or may stabilize the trimeric structure of TNF-α, and therefore may prolong its bioactivity. TNF-α release would lead to a subsequent induction of TNFRs production as a part of normal regulatory feedback mechanism.[8–10] Increased levels of TNFRs have been reported in serum and bronchoalveolar lavage fluid (BALF) of sarcoidosis subjects.[11–14] Recent studies have suggested that serum sTNFRII level assessment may serve as a prognostic factor of a chronic sarcoidosis course. The analysis of sTNFRII concentration in BALF and serum may be also useful in treatment planning.[13]

The studies on TNFRs as an activation marker concentrate on a soluble form of the receptors. So far the investigations have been focused on the level of sTNFRs in peripheral blood (PB) serum and BALF cells culture supernatant (CCS) of sarcoidosis patients. But the expression of the receptors on T cells from different specimens of patients with sarcoidosis was only fragmentary analyzed.

The aim of our study was to assess the level of sTNFRs in PB serum and BAL CCS, and to determine the receptor expression on BALF and PB cells in sarcoidosis patients with different clinical presentation, radiological stage, and clinical outcome of the disease.

Material and methods

Study population

The study population comprised 49 Polish Caucasian patients (the mean age of 39.3 ± 11.7 years; 25 female and 24 male subjects, all nonsmokers) with newly diagnosed sarcoidosis. The diagnosis of sarcoidosis was established using defined criteria including histopathological confirmation. None of the patients received steroid therapy or other immunosuppressive agents before bronchoscopy. Löfgren syndrome (fever, bilateral hilar adenopathy, erythema nodosum, and arthralgia; in accordance with ATS/ERS/WASOG guidelines) was manifested in 17 patients (the mean age of 35.4 ± 9.14 years).

Twenty-seven patients (the mean age of 35.6 ± 11.7 years) showed bilateral hilar lymphadenopathy in chest X-ray examination (radiological stage I). In 22 patients (the mean age of 41.4 ± 11.8 years), interstitial infiltration with or without hilar lymphadenopathy (stage II or III) were demonstrated. We made a distinction between the radiographic stage I with self-limiting clinical course, and the stage II or III with high probability of disease progression.

The assessment of the disease included clinical features, chest X-ray and computed tomography, lung function tests, abdomen ultrasonography, bronchoalveolar lavage (BAL), and routine blood tests. The follow-up examinations (clinical tests, lung function tests, chest X-ray, and computed tomography) were performed every 3 months. On the basis of 1- to 2-year follow-up (the mean period of 20.23 ± 3.67 months), the patients were divided into three groups: the group with spontaneous remission (the remission group consisted of 22 patients), the group with long-lasting disease without a need of treatment (the stable disease group—12 patients), and the group with disease progression and a need of steroid treatment (the progression group—15 patients). The decision on steroid therapy initiation and

inclusion to the progression group was based on the presence of severe systemic symptoms, lung function deterioration, and disease progression, as revealed on chest X-ray or CT scans. The remission was defined as spontaneous recede of clinical symptoms, improvement of lung function test results, and remission of chest X-ray changes.

The research project was approved by the Bioethic Committee of the Medical University of Lublin in accordance with the Guidelines for Good Clinical Practice.

Control group

The control group consisted of 10 nonsmoking, age- and sex-matched volunteers. None of them had a clinical and radiological evidence of lung disease or concomitant therapy. In morphology blood examinations, there was no evidence of inflammatory process. For the estimation of the percentage of T cells with TNFRs expression and serum levels of sTNFRI and sTNFRII, only blood samples were taken.

BAL procedure, and BALF cells preparation and culture

BAL was performed using Pentax EPM 3300 equipment. A bronchoscope was passed transorally under the adequate topical anesthesia with 4% lidocaine. The standard protocol of fiberoptic performance was applied to all patients.

BALF was obtained by standard washing of the middle lobe with seven 20-ml aliquots of pre-warmed sterile 0.9% saline. BALF specimens, except for the first bronchial fraction, were carefully mixed, strained through a double layer of nylon gauze, and centrifuged to obtain cell pellet. Cells were washed twice with phosphate-buffered saline (PBS). Cell pellet was resuspended in RPMI 1640 medium. Cells were counted in the Neubauer chamber, and used for flow cytometry analysis and culture.

10^6 cells per ml were cultured in RPMI 1640 medium supplemented with 2 mM L-glutamine and antibiotics in 6-well plates (Nunc, Denmark) for 24 h at 37 °C under 5% CO_2. CCS was centrifuged at 400*g* and stored at −80 °C for cytokine level measurement.

PB mononuclear cells preparation and cytometric analysis

Blood samples were taken before bronchoscopy. Lymphoprep (Nycomed, Norway) gradient centrifugation was used to separate PB mononuclear cells. Cells were collected and washed twice in PBS. Serum samples were also collected and cryopreserved at −80 °C for further ELISA performance.

Three-color flow cytometric technique was used in the analysis of PB and BALF lymphocyte subtypes. 10^6 cells/ml were incubated with different sets of fluorochrome-labeled monoclonal antibodies. Simultest (Becton Dickinson, USA), PE-conjugated monoclonal antibody (mAb) specific for the type I TNF receptor (CD120a) and the type II TNF receptor (CD120b; Caltag Laboratories, USA), FITC-conjugated mAb anti-CD4 antigen, and PerCP-conjugated mAb anti-CD8 antigen (Becton Dickinson, USA) were used. Cells were incubated for 20 min at room temperature and immediately analyzed in the flow cytometer FACS Calibure (Becton Dickinson, USA). The mean fluorescence intensity (MFI) of antigens was evaluated using the CellQuest software (Becton Dickinson, USA).

Assessment of sTNFRI and sTNFRII in PB serum and BALF CCS

The determination of soluble TNFRI and TNFRII levels was performed using enzyme-linked immunosorbent assay (ELISA) with the commercial Quantikine Human sTNFRI and sTNFRII kit (R&D Systems, USA) and the EL_X 800 equipment (Bio-Tek Instruments, USA).

Statistical analysis

The data are presented as the mean ± SEM. The Pearson χ^2 analysis was used to test the differences between the groups of patients depending on the gender. To evaluate statistical significance, the *U* Mann–Whitney test was used for the comparison of unpaired group data. The Wilcoxon matched pairs test was applied to compare two variables. In correlation observations, the Spearman correlation test was used. A level of $P<0.05$ was assigned as significant.

Results

Löfgren syndrome was diagnosed in 34.7% of patients; 48% of female subjects and only 20.8% of male patients had acute sarcoidosis. Löfgren syndrome was significantly more frequent in female patients than in male subjects ($\chi^2 = 3.99$, $P<0.05$). Löfgren syndrome patients were also significantly younger compared to subjects without acute symptoms of sarcoidosis ($P<0.05$). The distribution of gender between patients with stage I, II, or III of sarcoidosis showed no significant differences ($\chi^2 = 1.65$, $P = 0.199$) (Table 1).

Patients' gender and age had no influence on the immunological parameters analyzed in BALF and PB.

Table 1 Clinical characteristic of examined patients.

Characteristics	Patients
Subjects	$N = 49$
Subjects with Löfgren syndrome	$N = 17$ (34.7%)
Female/male	$N = 25/24$
Age (years)	39.3 ± 11.7
Chest X-ray stages I and II/III	$N = 27/22$ (55.1/44.9%)
Clinical outcome	
Remission	$N = 22$ (44.9%)
Stable disease (*n*, %)	$N = 12$ (24.5%)
Progression (*n*, %)	$N = 15$ (30.6%)
Lung function parameters	
VC % pred	91.92 ± 12.45
FEV_1% pred	85.83 ± 14.44
FEV_1/VC %	77.39 ± 9.69
TLC % pred	89.36 ± 12.64
DL_{CO} % pred	93.88 ± 14.79

Table 2 BALF immunological analysis in patients with different manifestation of sarcoidosis.

	Sarcoidosis patients	Löfgren syndrome patients	Patients without Löfgren syndrome	Patients in sarcoidosis stage I	Patients in sarcoidosis stage II or III
Lymphocytes (%)	42.56±20.18	43.93±20.78	41.03±19.33	39.19±20.14	45.18±19.14*
Macrophages (%)	52.82±20.72	53.08±22.24	53.69±19.71	56.95±20.78	49.32±19.49*
T lymphocytes (%)	90.63±4.91	92.40±3.33	89.92±5.28*	90.81±4.47	90.53±5.43
B lymphocytes (%)	1.20±1.11	0.82±0.50	1.33±1.19	1.18±0.80	1.18±1.30
NK cells (%)	4.07±3.28	2.72±1.17	4.65±3.68**	3.44±2.49	4.71±3.89
Th lymphocytes (%)	74.02±11.24	80.17±7.28	71.48±11.68**	75.60±11.67	72.45±10.88
Tc-s lymphocytes (%)	15.55±9.83	10.71±4.99	17.49±10.72*	13.78±9.87	17.22±9.83
CD4:CD8 ratio	7.23±5.23	9.77±6.06	6.52±5.51**	9.12±7.00	5.86±3.79
Activated T cells HLA DR+ (%)	54.33±14.36	57.08±217.29	52.45±14.48	49.88±14.60	58.55±13.46**

*$P<0.05$, **$P<0.01$.

Table 3 BALF immunological analysis in sarcoidosis patients with different disease course.

	Remission group	Stable disease group	Progression group
Lymphocytes (%)	40.0±20.5	43.59±16. 5	48.6±17. 7**
Macrophages (%)	56.3±21.7	50.6±17.3	47±19**
T lymphocytes (%)	91±3.4	91.3±3.5	91.9±5.9
B lymphocytes (%)	0.95±0.64	1.1±0.7	0.9±1.0
NK cells (%)	3.1±1.7	3.6±1.8	4.3±4.4
Th lymphocytes (%)	75.76±10.60	78.6±7.7	75±9.0
Tc-s lymphocytes (%)	14.44±9.2	11.1±6.3	17.1±9.7
CD4:CD8 ratio	7.43±4.57	9.77±5.45	5.76±3.0*
Activated T cells HLA DR+ (%)	54.2±15.2	56.4±17.2	61.0±11.5*

*$P<0.05$, **$P<0.01$ remission vs. progression.

In Löfgren syndrome patients, the percentage of T lymphocytes and Th CD4+ lymphocytes in BALF was significantly higher ($P<0.05$ and $P<0.005$, respectively) compared to patients without acute symptoms of the disease. In this group of patients, the CD4:CD8 ratio had the highest value ($P<0.01$). The percentage of BALF cytotoxic-suppressor CD8+ T lymphocytes and NK cells was significantly lower ($P<0.05$ and $P<0.005$, respectively) in Löfgren syndrome patients than in other subjects. In patients with hilar lymphadenopathy, the value of BALF CD4:CD8 ratio was only slightly higher ($P=0.056$), and the percentage of activated T lymphocytes HLA DR-positive was significantly lower ($P<0.01$) compared to patients with interstitial infiltrates (Table 2). Progression group patients had a significantly lower value ($P<0.05$) of BALF CD4:CD8 ratio and a significantly higher percentage of activated HLA DR-positive T lymphocytes ($P<0.05$) compared to remission group subjects (Table 3). Basic immunological parameters in PB had similar values in all examined groups.

Assessment of sTNFRI and sTNFRII in PB serum and BALF CCS

The level of sTNFRI in PB serum was similar in sarcoidosis patients (629.95±314.94 pg/ml) and in healthy subjects (655.14±164.28 pg/ml). But the concentration of serum sTNFRII was significantly higher in sarcoidosis patients (3454.12±1391.88 pg/ml) compared to healthy subjects (1601.21±360.23 pg/ml; $P<0.0001$). The level of soluble TNFRs in PB serum and BALF CCS was similar in all examined groups of sarcoidosis patients. However, we observed that patients without acute disease symptoms and patients with parenchymal infiltrates in the lung showed a tendency to higher levels of sTNFRs in PB serum and lower levels of sTNFRs in BALF CCS compared to subjects with Löfgren syndrome and hilar lymphadenopathy (Table 4, Fig. 1).

The level of sTNFRs in PB serum and BALF CCS was also similar in patients with different clinical course of sarcoidosis (Table 5). But we noticed a tendency to higher

Table 4 sTNFRs level in different specimens of sarcoidosis patients.

	Sarcoidosis patients	Löfgren syndrome patients	Patients without Löfgren syndrome	Patients in sarcoidosis stage I	Patients in sarcoidosis stage II or III
sTNFRI level in PB serum (pg/ml)	629.95±314.94	573.38±332.63	660.01±306.24	608.04±348.26	650.99±284.95
sTNFRI level in BALF CCS (pg/ml)	27.11±29.49	28.43±26.01	26.42±31.64	29.38±23.01	24.31±36.52
sTNFRII level in PB serum (pg/ml)	3454.12±1391.88	3373.38±1263.86	3497.01±1473.04	3325.01±1374.26	3578.06±1425.52
sTNRII level in BALF CCS (pg/ml)	178.51±183.45	199.94±217.29	167.36±167.02	208.88±207.68	140.98±145.60

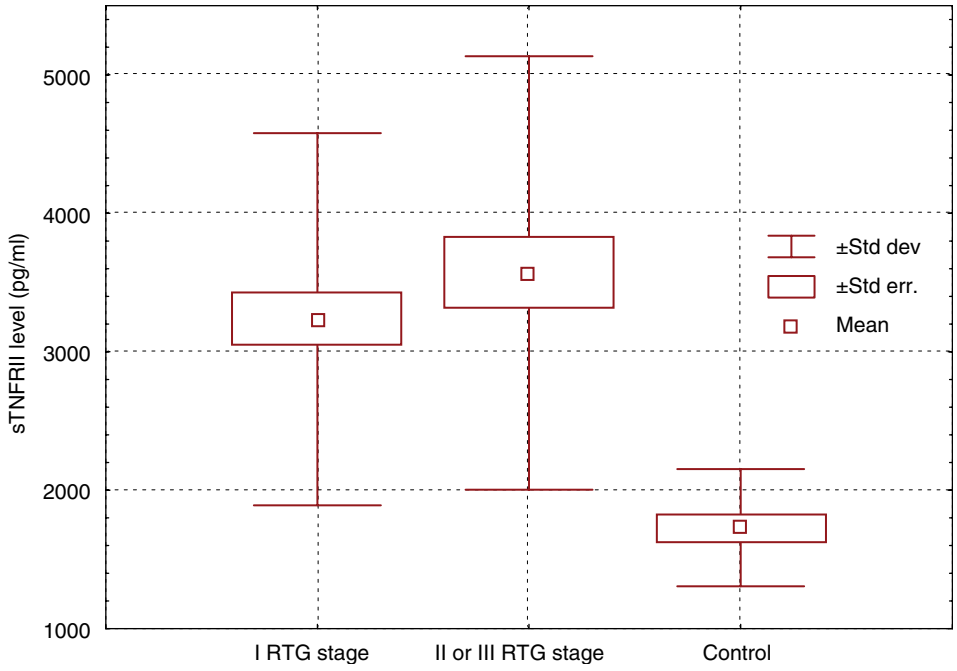

Figure 1 Serum sTNFRII level in patients with different radiological stages of sarcoidosis and in healthy subjects.

Table 5 sTNFRs level in sarcoidosis patients with different disease course.

	Remission group	Stable disease group	Progression group
sTNFRI level in PB serum (pg/ml)	537.54±312	660.28±331	683.16±283
sTNFRI level in BALF CCS (pg/ml)	27.28±24	39.33±49	18.74±21.8
sTNFRII level in PB serum (pg/ml)	3274.0±1190	3621.4±1645	3849.25±1681
sTNRII level in BALF CCS (pg/ml)	223.1±233	163.8±134	89.13±66

levels of sTNFRs in BALF CCS and lower levels of sTNFRs in PB serum of regression patients compared to patients with further disease progression (Table 4, Fig. 2).

In serum specimens of sarcoidosis patients, there was a nonsignificant positive correlation between sTNFRI and sTNFRII level ($R = 0.19$, $P = 0.145$). BALF CCS sTNFRI correlated significantly positively with sTNFRII levels in the same specimen ($R = 0.52$, $P > 0.005$).

In all sarcoidosis patients, serum sTNFRII concentration correlated positively with the percentage of BALF lymphocytes ($R = 0.28$, $P = 0.055$), the percentage of CD4+ cells ($R = 0.31$, $P < 0.05$), and the CD4:CD8 ratio ($R = 0.36$, $P < 0.05$). A significant negative correlation between TNFRII level in blood serum and the percentage of BALF macrophages ($R = -0.29$, $P < 0.05$) as well as BALF CD8-positive lymphocytes ($R = -0.36$, $P < 0.01$) was also found. TNFRI

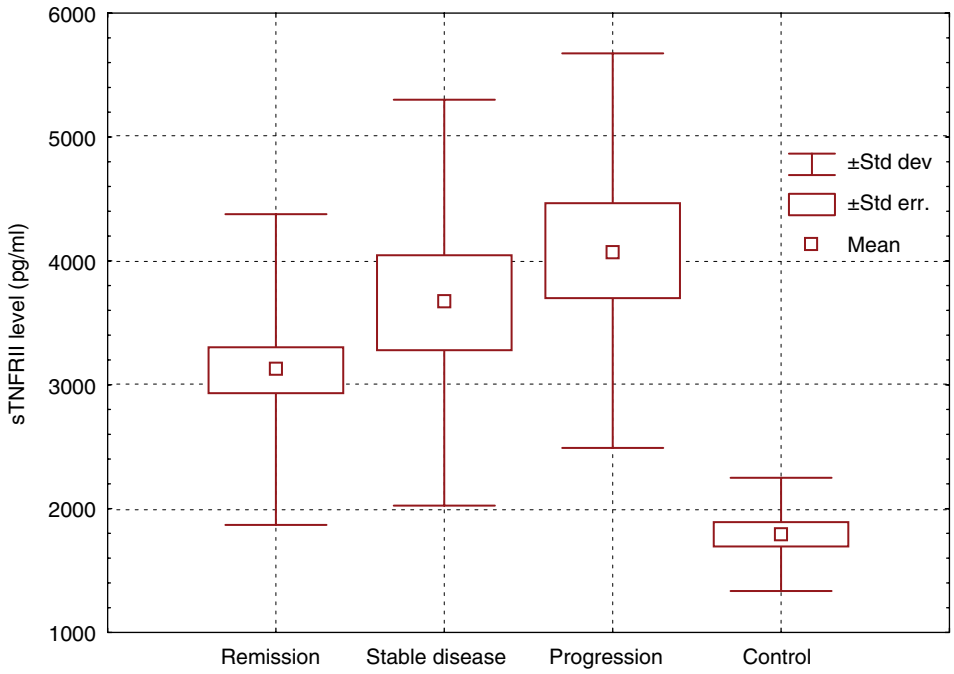

Figure 2 Serum sTNFRII in sarcoidosis patients with different course of the disease and in healthy subjects.

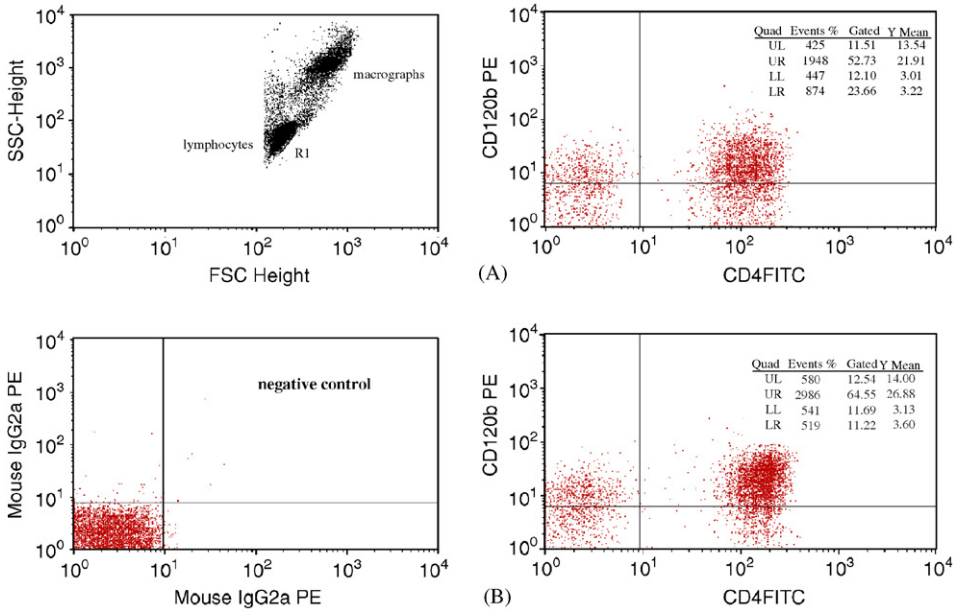

Figure 3 Flow cytometric analysis of CD120b expression on BALF CD4+ cells. (A) Löfgren syndrome patients with low CD120b expression on CD4+ cells. (B) Patients without acute symptoms of sarcoidosis with high CD120b expression on CD4+ cells.

and TNFRII levels in BALF CCS correlated significantly negatively with the percentage of activated T lymphocytes with HLA DR antigen ($R = -0.5$, $P < 0.001$).

TNFRI (CD120a) and TNFRII (CD120b) expression on BALF and PB T lymphocytes

In sarcoidosis patients and healthy subjects, more than 80% of BALF and PB T lymphocytes expressed both CD120a and CD120b.

The percentage of BALF double-positive CD8+/CD120a+ cells and CD8+/CD120b+ cells was the lowest in Löfgren syndrome patients and in patients with lymphadenopathy. Patients with radiological stage I of sarcoidosis were characterized by a high percentage of double-positive CD4+/CD120a+ ($P < 0.05$). In Löfgren syndrome patients, the expression of CD120a and CD120b on BALF CD4+ cells was slightly lower compared to patients without acute symptoms of the disease (Fig. 3). We found no significant differences between patients with remission, stable disease, and sarcoidosis progression as far as the percentage of

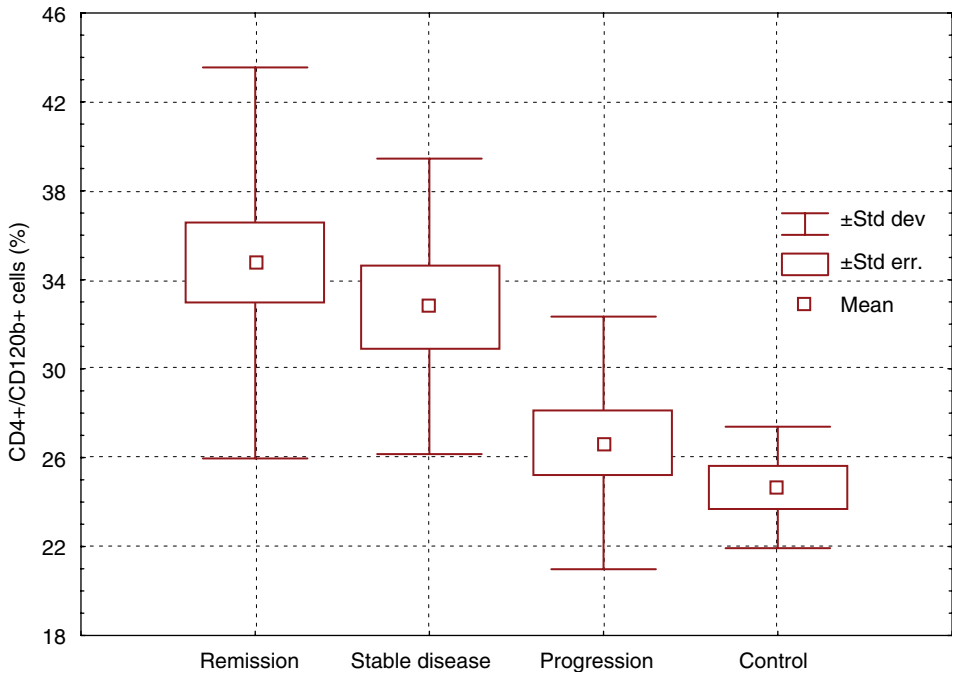

Figure 4 PB CD4+/CD120b+ cell percentage in sarcoidosis patients with different disease course and in healthy subjects.

double-positive BALF cells and expression of examined antigens was concerned.

The percentage of PB double-positive CD4+/CD120b+ cells was significantly higher ($P<0.005$) in sarcoidosis patients ($32.41\pm8.86\%$) than in healthy subjects ($24.66\pm2.72\%$). Most interestingly, the percentage of double-positive CD4+/CD120a+ and CD4+/CD120b+ cells in PB was significantly higher ($P<0.005$) in patients with radiological stage I sarcoidosis ($34.19\pm7.51\%$ and $34.95\pm7.07\%$, respectively) than in subjects with RTG stage II or III ($27.74\pm8.56\%$ and $29.57\pm9.89\%$, respectively). The percentage of PB double-positive CD4+/CD120b+ cells was significantly higher in patients with stable disease ($32.85\pm7.6\%$, $P<0.05$) and disease remission ($35.44\pm9.0\%$, $P<0.001$) compared to healthy subjects. No significant differences were observed between patients with sarcoidosis progression ($26.66\pm5.7\%$) and healthy subjects (Figs. 4 and 5). We also found that patients with spontaneous remission had a significantly higher percentage of double-positive CD4+/CD120a+ ($34.35\pm9.17\%$) and double-positive CD4+/CD120b+ ($35.44\pm9.0\%$) cells than patients with sarcoidosis progression ($25.7\pm5.4\%$ and $26.66\pm5.7\%$, respectively).

In PB, CD120b antigen expression on CD4+ and CD8+ cells was significantly higher in sarcoidosis patients ($P<0.001$ and $P<0.01$) than in healthy subjects. No significant differences between sarcoidosis patients with different clinical outcome were observed when comparing the expression of CD120a and CD120b on PB CD4+ and CD8+ cells.

The percentage of BALF double-positive CD4+/CD120a+, CD4+/CD120b, and CD8+/CD102b cells correlated significantly positively with the percentage of PB cells ($R=0.3$, $P<0.05$). Also, a positive correlation between the expression of CD120a and CD120b antigens on BALF and PB cells was found ($R=0.6$, $P<0.0001$).

The level of sTNFRI and sTNFRII in serum correlated negatively with the percentage of BALF double-positive CD4+/CD120a+ and CD4+/CD120b+ cells ($P<0.05$). However, sTNFRI and sTNFRII levels in BALF CCS correlated positively with the expression of CD120a and CD120b on BALF and PB T cells ($P<0.005$).

Discussion

Our results demonstrated that serum sTNFRs level estimation may be highly useful in the prognosis of sarcoidosis course. Therefore, our results are partly in accordance with the findings of Nakayama et al. as well as Ziegenhagen et al.

Nakayama et al.[14] measured plasma sTNFRI and and sTNFRII levels in 19 patients with active sarcoidosis. The levels of two receptor types were significantly increased in sarcoidosis group. There was also a significant correlation between the receptors' level and the percentage of BALF lymphocytes, but not the CD4/CD8 ratio. Nakayama et al. found no differences in receptor plasma levels between patients at different radiological stages and different clinical outcome.

Ziegenhagen et al.[13] determined sTNFRI and sTNFRII serum levels in 49 patients with sarcoidosis. Serum levels of two receptors were significantly higher in sarcoidosis group compared to control group, but only sTNFRII was significantly increased in sera of patients with radiological stages II and III. The highest sTNFRII levels were observed in patients with disease progression, whereas the lowest in patients with spontaneous remission, and the differences were close to significance. sTNFRI and sTNFRII correlated positively with the percentage of BALF neutrophils and eosinophils, but not with the percentage of lymphocytes or

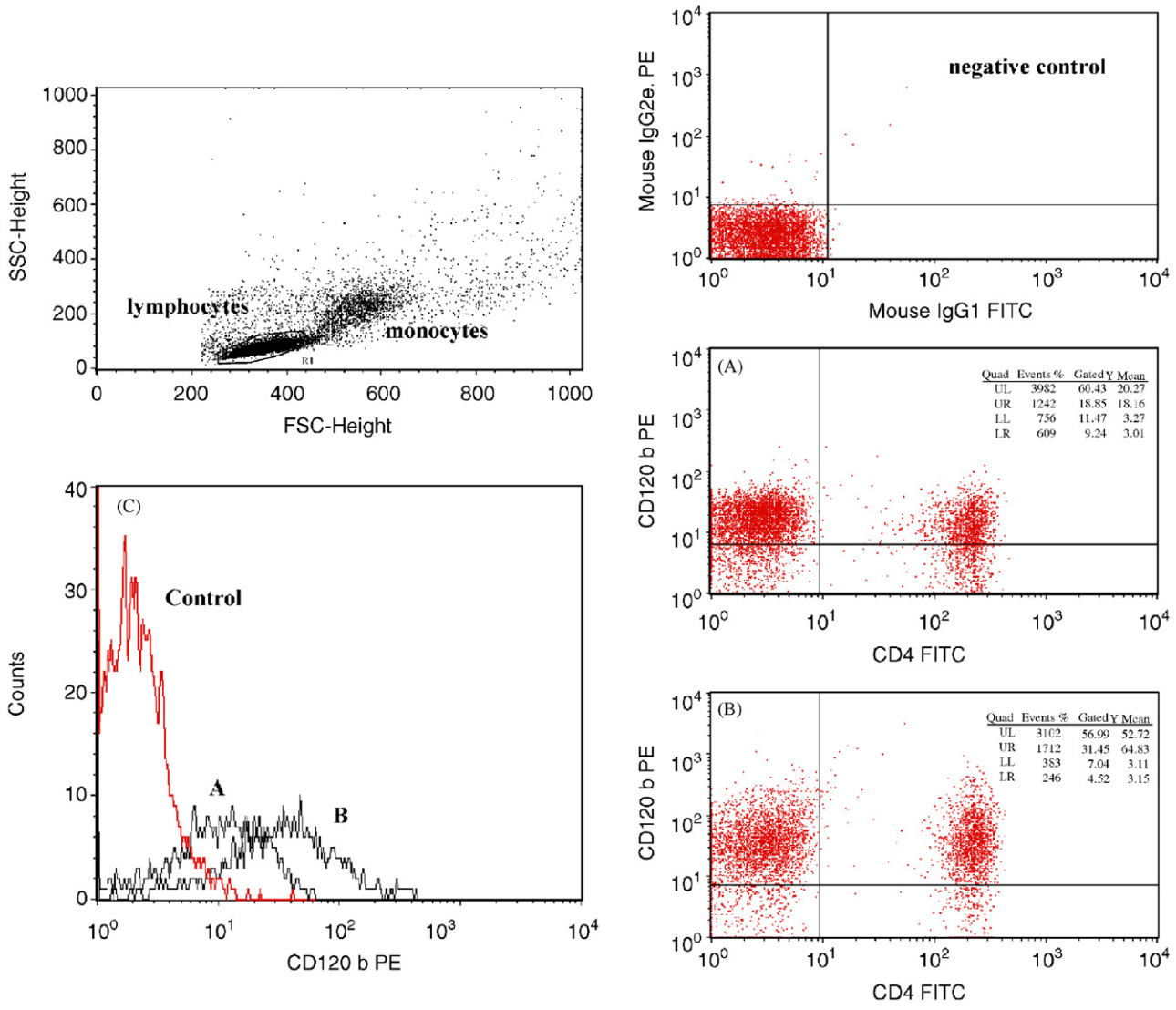

Figure 5 Flow cytometric analysis of CD120b expression on PB CD4+ cells. (A) Low percentage of double positive CD4+/CD120b+ cells characteristic of patients with RTG stage I of sarcoidosis with further disease remission. (B) High percentage of double positive CD4+/CD120b+ cells in patients with RTG stage III of sarcoidosis with further disease progression. (C) Comparison of CD120b expression on CD4+ cells of patients A and B.

macrophages. Ziegenhagen and co-workers have suggested that the level of two sTNFRs types is elevated in serum specimens of sarcoidosis patients with active disease, but only sTNFRII seems to be useful in the monitoring of disease inflammatory activity. Inclusion criteria for the examined group of patients may explain the differences between Ziegenhagen's and our results. Ziegenhagen et al. excluded Löfgren syndrome patients ("classic acute presentation"), assuming good clinical prognosis in that group of patients. On the other hand, the investigators included a large group of patients with severe and chronic disease. On the contrary, our examined group consisted of newly diagnosed patients, with a high percentage of Löfgren syndrome cases.

Also, our results demonstrated a higher concentration of sTNFRII, but not sTNFRI, in serum samples of sarcoidosis patients compared to the control group. We also found a tendency to higher sTNFRs levels in patients with radiological stage II or III, and patients with further progression of sarcoidosis compared to patients with radiological stage I, and patients without disease progression. sTNFRs level correlated with other immunological prognostic factors such as the percentage of BALF lymphocyte, and activated HLA DR+ T cells.

Dai et al.[15] measured the production of sTNFRs in alveolar macrophages (AM) culture supernatants from 10 patients with sarcoidosis and nine control subjects. Compared to the control group, spontaneous and lipopolysaccharide (LPS)-stimulated production of sTNFRs and TNF-α was significantly increased in sarcoidosis patients. The concentration of two sTNFRs types, but mainly of sTNFRII, was closely related to that of TNF-α. LPS-induced increase was 1.5-fold for sTNFRI, at least four-fold for sTNFRII, and at least 25-fold for TNF-α in all examined patients. These results indicate that AMs can release two sTNFRs in relation with TNF-α. sTNFRII may be more liable to shedding than sTNFRI. The authors concluded that both sTNFRI and sTNFRII may be involved in the

pathogenesis of sarcoidosis, possibly as counter-regulators of TNF-α.[15]

Similar results were obtained by Hino and co-workers.[16] They evaluated BALF sTNFRs level in 13 sarcoidosis patients and 18 control subjects using ELISA. Type II, but not type I sTNFR in BALF was significantly elevated in sarcoidosis patients compared to healthy nonsmokers. Although the levels of sTNFRI in BALF of sarcoidosis patients were not correlated with any cellular profile of BALF, the concentrations of type II receptor correlated significantly with the number of BALF lymphocytes. In their study, Hino et al. included, however, six sarcoidosis patients who received corticosteroid therapy.[16]

We were not able to assess sTNFRI and sTNFRII on BALF CCS in the control group, but we found a tendency to higher levels of sTNFRs in BALF CCS in the group of Löfgren syndrome patients, radiological stage I, and spontaneous remission compared with the group of patients without acute symptoms, radiological stage II/III, and patients with disease progression. Moreover, we showed a significant correlation between sTNFRs levels in PB and BALF as well as the dependence between sTNFRs concentration and TNFR expression on T cells in different specimens. It may contribute to the progression of systemic immunologic response in sarcoidosis, and may show that the cytokine network could play an important role in sarcoidosis development and prolongation.

Armstrong and co-workers[11] have attempted to explain that phenomenon. The investigators found a reduced TNF-α bioactivity in AM supernatants derived from sarcoidosis patients with stage I compared to those with stage II/III and the control group. That contrasted with increased sTNFRI levels in AM culture supernatants derived from subjects with stage I compared to patients with stage II/III and healthy subjects. The authors concluded that the inhibition of TNF-α bioactivity by increased sTNFRs levels may be higher in patients with radiological stage I than in those with stage II or III, and this may represent a homeostatic protective mechanism against an excessive TNF production characteristic for chronic inflammation.[11]

The hypothesis concerning the role of sTNFRs in pathological process limitation may become useful in the development of new therapeutic strategies of sarcoidosis management. Corticosteroids are usually effective in blocking TNF-α cell release. Other agents that nonspecifically inhibit TNF-α release include methotrexate, azathiopryne, and pentoxiphylline. Specific TNF-antagonizing biological agents such as infiximab, etanercept, and adalimumab are tested in sarcoidosis patients, with various results.[17–20] Utz et al.[17] showed that in patients with progressive stage II or III of pulmonary sarcoidosis, etanercept was frequently associated with an early or late treatment failure. The authors concluded that neither absolute levels of TNF-α nor TNF-α activity in blood serum, BALF, or supernatant of alveolar macrophages culture were able to predict which patients would respond to etanercept.

Little is known about the expression of two TNFRs presented on BALF and PB cell surface in sarcoidosis. Dai et al.[21] found the expression of Fas receptor (FasR) and TNFRI on the higher percentage of AM in sarcoidosis in comparison with control subjects. FasR and TNFRI are recognized as apoptotic signaling receptors. The authors suggested that alveolar macrophages of sarcoidosis patients undergo more apparent apoptosis than those of control subjects.[21]

Using a flow cytometry technique, Gaede et al.[22] showed that CD120a is predominantly expressed on alveolar macrophages, and CD120b was mainly expressed on lymphocytes, macrophages, and PB mononuclear cells in the group of patients with different pulmonary disorders. The authors found 0.8% CD120a-positive blood lymphocytes and 36.23% CD120b-positive blood lymphocytes in the group of four patients. BALF cells were collected from 14 patients; 4.13% and 25.48% of BALF lymphocytes were CD120a- and CD120b-positive.[22] Agostini and co-workers[12] could not detect the CD120a antigen on T lymphocytes and the CD120b antigen on CD4-positive T cells of sarcoidosis patients. Recently, Chen and co-workers[23] found a higher expression of TNFRII on lymphocytes from patients with hypersensitivity pneumonitis than in the control group, and patients with interstitial pulmonary fibrosis.

In our group of patients, more than 80% of BALF and PB lymphocytes expressed both CD120a and CD120b antigens. An increased expression provides more sites for TNF-α binding, and may enhance immunological reaction in sarcoidosis contributing to alveolitis and granuloma formation. Differences in TNFRs expression may be due to the influence of different levels of various cytokines: IFN-γ,[24] IL-2,[25] IL-1, and TNF-α.[26] Therefore, in order to explain the relationship, precise investigations of a whole cytokine network is required.

The measurement of PB double-positive CD4+/CD120b+ cell percentage is interesting. High percentage of these cells seems to be characteristic of sarcoidosis patients with further disease remission. These findings could indicate the role of these cells in downregulation of a cell-mediated immunological response. Immunophenotyping of PB cells may also serve as a convenient prognostic marker of sarcoidosis.

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